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Circadian and seasonal variations in the metabolism of carbohydrates in *Aegla ligulata* (Crustacea: Anomura: Aeglidae)

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Abstract

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The aim of this study is to evaluate the effect of circadian and seasonal variations on the metabolism of carbohydrates in different tissues of the freshwater anomuran *Aegla ligulata* Bond-Buckup and Buckup, 1994. Samples of *A. ligulata* were collected monthly from August 1999 to August 2000 in Tainhas, São Francisco de Paula, RS, Brazil, at 0600 h, 1200 h and 1800 h. Samples of haemolymph and tissues (hepatopancreas, gills and muscle) were taken to determine glucose and glycogen levels. Data indicated the presence of high levels of haemolymphatic glucose, especially in spring, and we also found circadian differences between males and females. These variations seem to be related to the reproductive period of the species, food availability and the degree of environmental exploration. These factors lead to different metabolic adjustments in distinct species of crustaceans.

Keywords Crustacea, Anomura, Aeglidae, metabolism

Introduction

Crustaceans are exposed to many environmental variables that follow annual and daily cycles differing with geographical region, and which cause behavioural, feeding and metabolic alterations. Study of intermediate metabolism in crustaceans has shown high inter-and intra-specific variability, which makes it difficult to determine a standard metabolic profile. This variability can occur because of several factors such as habitat, stage in the moult cycle, sexual maturity (especially in females), feeding state, food at hand and seasonality, since these factors determine differential metabolic response.

Glucose is the principal monosaccharide present in the haemolymph of crustaceans and it serves six main purposes: synthesis of mucopolysaccharides, synthesis of chitin, synthesis of ribose and nicotinamide adenine dinucleotide phosphate reduced (NADPH), the formation of pyruvate, and the synthesis of glycogen (Hochachka et al., 1970; Chang and O'Connor, 1983; Herreid and Full, 1988).

The main glycogen reserves in crustaceans are the muscle, the hepatopancreas, the branchiae and the haemocytes. The storage place of this polysaccharide varies according to the species (Johnston and Davies, 1972; Herreid and Full, 1988). The absence of a central glycogen deposit seems to be an adaptation of several classes of animals to changes in environmental factors (Hochachka et al., 1970). The stored glycogen is utilized in molting, adaptation to hypoxia and/or anoxia, osmoregulation, growth, in the different stages of reproduction, and during fasting periods (Hu, 1958; Chang and O'Connor, 1983; Kucharski and Silva, 1991a, 1991b; Oliveira and Da Silva, 2000; Oliveira et al., 2001a, b).

Since very little is known about the physiology of *Aegla*, the aim of this study is to evaluate the effect of circadian and seasonal variations on the metabolism of carbohydrates in different tissues of the freshwater anomuran *Aegla ligulata*.

Material and methods

Samples of *Aegla ligulata* were collected at 0600, 1200 noon and 1800 h one day, every month from August 1999 to August 2000 in the region of Tainhas, São Francisco de Paula, RS, Brazil. The animals were separated according to sex, samples of haemolymph were collected in the field with a syringe containing potassium oxalate (10%) as an anti-clotting agent. The

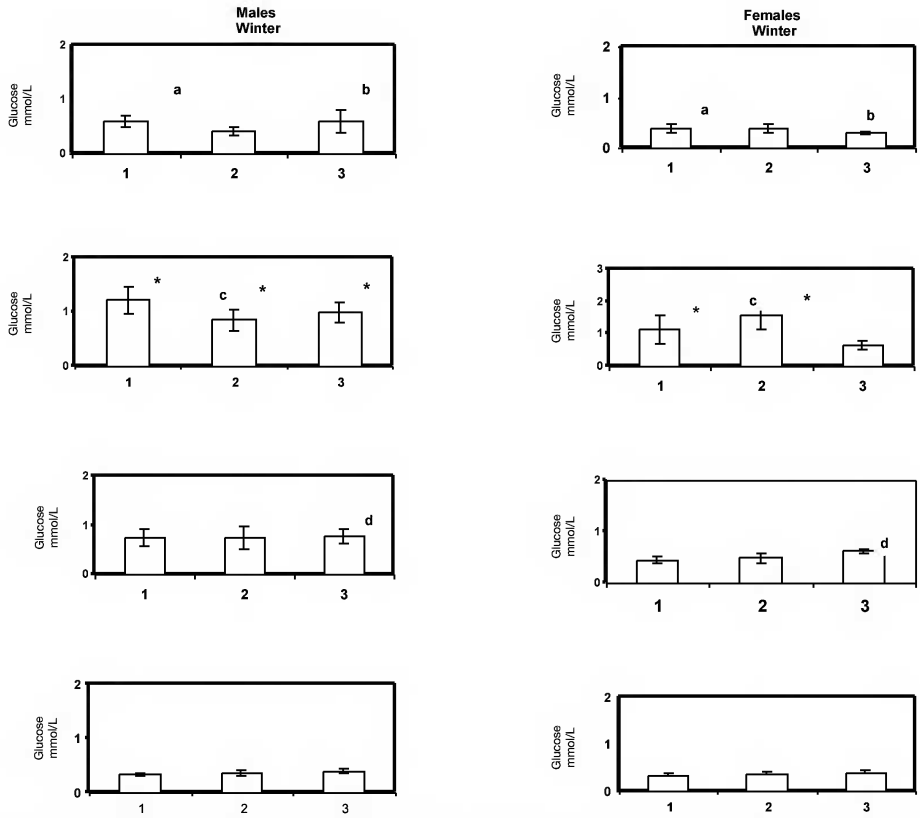

Figure 1. Circadian and seasonal variations of haemolymphatic glucose levels in *Aegla ligulata* Bond-Buckup and Buckup, 1994, males and females. Data are given as mean ± SEM. The number of animals at each point varied between 15 and 20. The same letter denotes significantly different means ($P< 0.05$). * denotes significantly different means of the spring (Sep, Oct and Nov), winter (Jun, Jul and Aug), summer (Dec, Jan and Feb) and autumn (Mar, Apr and May). Numbers 1, 2 and 3 stand for the collection times: 0600, 1200 and 1800 h, respectively.

animals and the haemolymph samples were frozen in the field. In the lab, the tissues (hepatopancreas, branchiae and muscle) were removed and grouped according to collection time. Tissue glycogen was extracted following Van Handel (1965) and determined to be glucose (enzymatic oxidase method) upon acid hydrolysis (HCl) and neutralisation (Na_2CO_3), and the results were expressed in mmol g^{-1}. The levels of haemolymphatic glucose were dosed according to the enzymatic oxidase method (Biodiagnóstica: enz-color glucose kit), and the results were expressed in mmol l^{-1}. The number of animals collected varied between 15 and 45 per season of the year (winter: June, July and August; spring: September, October and November; summer: December, January and February; autumn: March, April and May).

For the statistical analysis of the circadian and seasonal variations found, a one-way ANOVA test was used, followed by Tukey's comparison test. For the comparison between sexes, a t-Test for the independent samples was used. The significance level adopted was 5%, and the statistical analyses were carried out in the program Statistical Package for the Social Sciences (SPSS) for Windows. The Sigma Stat software was used to confirm parametrisation of the data.

Results and discussion

The concentrations of tissue glycogen and glucose in the haemolymph in this study were similar to those of other crustacean species, including those of the same genus (*Aegla platensis*) (Kucharski and Da Silva, 1991b; Oliveira et al., 2001b). The behaviour of such metabolic parameters, however, differs in relation to circadian and seasonal variations.

The levels of haemolymphatic glucose of males and females did not vary during the day (Fig.1). Males presented higher glycemic levels ($p<0.05$) than females at 1800 h in the summer, and at 0600 h and 1800 h in the winter. Females, however, had higher levels than males only at 1200 h in spring. Such findings suggest differences in exploration and/or feeding time for males and females. Studies on *A. ligulata*, developed by Bueno and Bond-Buckup (2001), have shown an increase both in feeding activity and repletion degree at 1800 h, regardless of season of the year. Furthermore, in the autumn months of March, April and May, no difference was found in males or females. In this period, May, Bueno and Bond-Buckup (2000) found a higher number of females with eggs in this species.

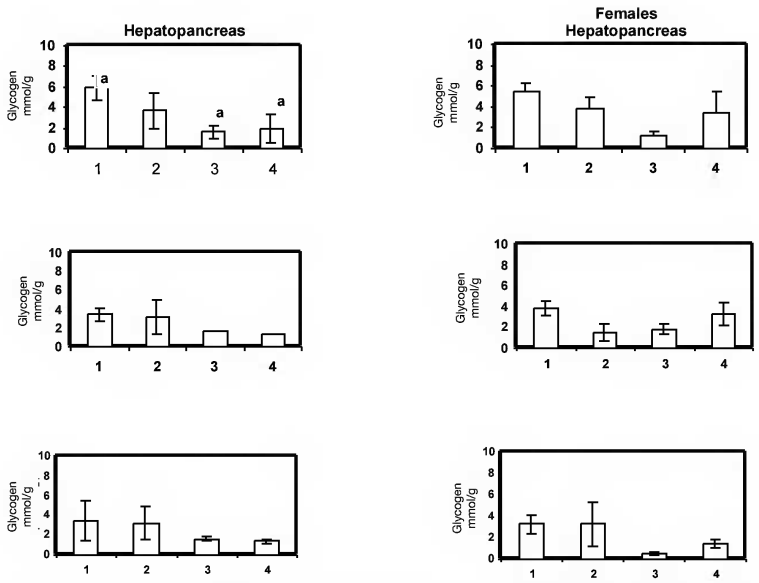

Figure 2. Seasonal variation of glycogen levels in tissues of males and females of *Aegla ligulata* Bond-Buckup and Buckup, 1994. Data are given as mean ± SEM. The number of animals in each point varied between 40 and 60. The same letter denotes significantly different means ($P < 0.05$). Numbers 1, 2, 3 and 4 stand for seasons: winter (Jun, Jul and Aug), spring (Sep, Oct and Nov), summer (Dec, Jan and Feb) and autumn (Mar, Apr and May), respectively.

In spring the highest concentrations of haemolymphatic glucose were found both in males and females; they were significantly higher than those found in the winter, summer and autumn. Similar results were found elsewhere, in the region of Taquara, for the crustacean *A. platensis* (Oliveira et al., 2001b). Bueno and Bond-Buckup (2001) working with *A. ligulata,* mentioned that food is more plentiful in the environment in spring and this secies presented higher feeding activity. The food items of *A. ligulata* varied according to season; in spring there is a predominant consumption of macrophytes and, in summer and autumn, insects are consumed in the same proportion. In winter, however, insects are the predominant food item. These results permitted Bueno and Bond-Buckup (2001) to characterize *A. ligulata* as an opportunistic omnivore. Haemolymphatic glucose is the result of influx of intestinal glucose, of the gluconeogenic pathway and utilisation of this hexose in different processes (Hu, 1958; Chang and O'Connor, 1983; Herreid and Full, 1988; Oliveira and Da Silva, 1997).

There were no variations during the day for glycogen levels in different tissues in males or females; for this reason data from different times were grouped for in the study of seasonality. No seasonal variations in tissue glycogen levels were found in females. The males in winter, however, showed hepatopancreatic glycogen levels 3 and 2.5 times as high ($p<0.05$) as those verified in summer and autumn, respectively (Fig. 2). In winter the exploratory activity of *Aegla* is reduced, and this fact is reflected by the difficulty of collection. In other crustaceans a shorter activity period and decreased metabolism have been observed, as well as a higher glycogen level in the hepatopancreas during winter, June–August (Kucharski and Da Silva, 1991b; Nery and Santos, 1993). This fact may account for the higher glycogen levels in the hepatopancreas during winter. Different results were found in females of *A. platensis* (Oliveira et al., 2001b). In this species seasonal variations were found in the levels of tissue glycogen, where the hepatopancreas showed higher values in autumn ($p<0.05$) than in other seasons and males did not show seasonal variation (Oliveira et al., 2001b).

The different tissues analysed seem to have the same capacity to store glycogen in both males and females (Fig. 2). According to Hochachka et al. (1970), this independence from a central deposit of glycogen seems to be an important adaptation of animals with an exoskeleton and open circulation, since their blood would flow slowly and under low pressure, leading to less effective distribution of glucose to the tissues. The circulatory systems are highly efficient and controlled in a complex manner, and cardiac outflow is not distributed equally among the vascular circuits during activity and hypoxia (McMahon, 2001). This adaptation would allow for animals to respond faster and more effectively to different environmental stresses. As the glycogen values in different tissues of both sexes were compared, no significant differences were found. It can be noted that such findings differ from those for a population of *Aegla platensis* (Oliveira et al., 2001b).

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